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Opposite effects of $GABA_B$ receptor antagonists on absences and convulsive seizures

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Abstract

In Wistar rats with spontaneous non-convulsive absence epilepsy, absence seizures were dose dependently suppressed by intraperitoneal administration of the $GABA_B$ receptor antagonists CGP 36742, 50–400 mg/kg, and CGP 56999, 0.25–0.75 mg/kg, and by bilateral microinjections of the same compounds into the lateral nuclei of the thalamus. In rats susceptible to audiogenic seizures, intraperitoneal administration of both $GABA_B$ receptor antagonists, at doses which suppressed absence seizures, facilitated the elicitation of sound-induced tonic seizures. In non-epileptic control rats, intraperitoneal injections of higher doses of CGP 36742 (800–2400 mg/kg) and CGP 56999 (3–6 mg/kg) induced delayed clonic convulsions, which were suppressed by pretreatment with baclofen. c-Fos protein was expressed after $GABA_B$ receptor antagonist-induced seizures in the cortex, hippocampus, amygdala, perirhinal and piriform cortex. Intra-cortical and hippocampal microinfusion of both $GABA_B$ receptor antagonists produced focal seizures. In conclusion, $GABA_B$ receptor antagonists suppress non-convulsive absence seizures by blocking thalamic $GABA_B$ receptors, while they induce convulsions in cortical and limbic structures. © 1997 Elsevier Science B.V.

Keywords: Epilepsy; Cortex; Hippocampus; Thalamus; c-Fos; $GABA_B$ receptor antagonist

1. Introduction

Generalized epileptic seizures may be classified into two broad groups, the non-convulsive and the convulsive types. The non-convulsive type, termed absence seizure, is characterized by arrest of behavior and disconnection from environmental inputs, associated with bilateral and synchronous cortical spike-and-wave discharges. At the end of the electroencephalographic discharge, the electroencephalogram (EEG) and behavior return to 'normal' immediately. Absence seizures have a well-defined thalamo–cortical substrate, and there is no spread to other systems of the brain (Avoli and Gloor, 1994; Snead, 1994; Vergnes and Marescaux, 1994). Convulsive seizures are characterized either by clonus associated with high amplitude cortical spike and spike-and-wave discharges, or by tonic manifestations concomitant with a short cortical desynchronization. At the end of these seizures, gradual recovery precedes the EEG and behavioral normalization. Clonic seizures depend on forebrain networks, whereas tonic seizures originate within the brain-stem (Browning, 1994).

It is well established that the inhibitory neurotransmitter γ-aminobutyric acid (GABA) is involved in the control of generalized epileptic seizures. GABA mediates its action in the mammalian brain via at least two classes of receptors, $GABA_A$ and $GABA_B$ receptors, which differ in terms of pharmacological profile, mechanisms of transduction and regional distribution (Hill and Bowery, 1981; Dutar and Nicoll, 1988; Bowery, 1993). Activation of $GABA_A$ receptors, which are coupled directly to an ion-channel, results in an increase in chloride conductance, inducing early, short duration inhibitory post-synaptic potentials. $GABA_B$ receptors, present in most brain structures, are located in the post-synaptic membrane and on presynaptic axonal terminals (Nicoll and Dutar, 1990; Bowery and Pratt, 1992; Thompson et al., 1992; Bowery, 1993; Deisz et al., 1993). They are coupled to their effectors via G-proteins. Activation of post-synaptic $GABA_B$ receptors mediates a late long-lasting inhibitory post-synaptic potential, while presynaptic $GABA_B$ receptors regulate the re-

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lease of several neurotransmitters and neuropeptides from nerve terminals (Pittaluga et al., 1987; Morrisett et al., 1991; Bowery, 1993; Pende et al., 1993; Potier and Dutar, 1993). Blockade of presynaptic receptors increases the release of glutamate and GABA from cortical slices (Waldmeier et al., 1994).

Convulsive and non-convulsive seizures differ in their pharmacological reactivity to GABAergic drugs. Non-convulsive absence seizures are aggravated by the administration of drugs activating $GABA_A$ receptors (Vergnes et al., 1984; Snead, 1994). In contrast, $GABA_A$ receptor agonists, as well as drugs which allosterically modulate the receptor–channel complex, are therapeutically active against convulsive seizures, whilst blockade of $GABA_A$ receptors generates convulsive seizures (Möhler et al., 1997). Similarly, the $GABA_B$ receptor agonist baclofen increases the number and duration of spike-and-wave discharges in several models of absence seizures (Marescaux et al., 1992a,b; Snead, 1994), but is reported to have antiepileptic activity in convulsive seizures (Ault and Nadler, 1983).

Consequently, such opposite effects on absences and on convulsive seizures may also be expected with $GABA_B$ receptor antagonists. Indeed, the $GABA_B$ receptor antagonist CGP 35348, γ-aminopropyl–(diethoxymethyl)–phosphinic acid, suppresses absence seizures in almost all rodent models (Hosford et al., 1992; Marescaux et al., 1992a,b; Snead, 1992), but in contrast to the highly convulsant $GABA_A$ receptor antagonists, $GABA_B$ receptor antagonists are not fully recognized for their convulsant effects.

New generations of potent $GABA_B$ receptor antagonists are now available and two representative compounds were used in the present study: the low-affinity antagonist CGP 36742, γ-aminopropyl-*n*-butyl-phosphinic acid, and the high-affinity antagonist CGP 56999, [3[[1-(*R*)-(3-carboxyphenyl) ethyl] amino] 2 (*S*)-hydroxy-propyl] cyclohexyl–methyl-phosphinic acid (Bittiger et al., 1992; Waldmeier et al., 1994). The anti-absence and the convulsive effects of systemic or focal administration of these compounds were investigated. In order to localize the brain structures activated by systemic administration of these $GABA_B$ receptor antagonists and by the resultant seizures, the induced expression of c-Fos protein was determined by immunocytochemistry (Dragunow and Faull, 1989).

2. Materials and methods

2.1. Drugs

Representative compounds from each of two generations of $GABA_B$ receptor antagonists were used in the present study: CGP 36742, a low-affinity (binding assay $IC_{50} = 35\ \mu M$) antagonist, and CGP 56999, a high-affinity (binding assay $IC_{50} = 2$ nM) antagonist (Froestl et al., 1992; Bittiger et al., 1993; Waldmeier et al., 1994).

R-baclofen, CGP 36742 and CGP 56999 were kindly provided by Dr. W. Froestl (Ciba-Geigy, Basel, Switzerland). The drugs were dissolved in sterile saline and administered by intraperitoneal (i.p.), or by focal intracerebral injections.

2.2. Animals

Adult male Wistar rats (300–450 g) from three inbred strains selected in our laboratory were used. The animals were individually housed, allowed ad libitum access to food and were kept under a 12 h light/dark cycle. Before the start of the experiments, they were tamed by daily handling.

2.2.1. Genetic Absence Epilepsy Rats from Strasbourg (GAERS)

GAERS exhibit spontaneous bilateral and synchronous high amplitude, 7–9 c/s spike-and-wave discharges concomitant with immobility and lasting 10–30 s. They occur at an average frequency of once per minute, when the rats are awake and inactive. These spike-and-wave discharges fulfil the requirements for an experimental model of generalized non-convulsive absence epilepsy (Vergnes and Marescaux, 1994).

2.2.2. Audiogenic seizures susceptible rats

Audiogenic seizures susceptible rats (AS) respond to a high-intensity sound by having a generalized convulsive seizure characterized by an episode of wild running followed by a tonic phase (Hirsch et al., 1994).

2.2.3. Control non-epileptic rats

Control non-epileptic rats (NE) are free of any spontaneous spike-waves and are insensitive to sound.

2.3. Surgery

Surgical procedures were performed under pentobarbitone anaesthesia (45 mg/kg i.p.) in a stereotaxic frame. Stereotaxic coordinates were adapted according to the Atlas of Paxinos and Watson (1986) and are given in mm, with lambda as reference.

For EEG recordings, all GAERS and NE rats were fitted with four single contact electrodes connected to a microconnector. The electrodes, made from stainless-steel dental screws, were bilaterally implanted over the frontal and parietal cortex.

For intra-cerebral microinjections, permanent stainless-steel guide cannulae (o.d. = 0.4 mm; i.d. = 0.3 mm) were positioned bilaterally above the thalamic relay nuclei in GAERS, and above the dorsal hippocampus or the fronto–parietal cortex in NE rats. The following coordi-

nates were used: thalamus AP = 5.0, ML = 2.0, DV = 4.5; hippocampus AP = 4.0, ML = 2.0, DV = 3.0; cortex AP = 7.0, ML = 2.0, DV = 1.0. Hippocampal and cortical cannulae were also coupled to the microconnector through an isolated wire in order to allow EEG recordings of the injected area. Stainless-steel stylets (o.d. = 0.27 mm) were placed in each guide cannula.

The guide cannulae and EEG electrodes were anchored to the skull, using retaining screws and were embedded in dental acrylic cement. All animals were then allowed one week recovery.

2.4. Anti-absence action

Each drug and each mode of administration was assigned to a group of 8 GAERS. In each group, all rats serving as their own controls were injected in a randomized order with the different doses of the drug under study, and with the saline solvent, with at least three days between injections.

Drugs were injected i.p. in a final volume of 2–4 ml/kg, depending on the doses used (CGP 36742, 50–400 mg/kg; CGP 56999, 0.25–1.5 mg/kg) and bilaterally in the relay nuclei of the thalamus in a volume of 0.5 μl/side (CGP 36742, 0.5–1 μg/side; CGP 56999, 0.1–0.2 μg/side). Intrathalamic injections were given to awake animals, gently hand held, by introducing stainless steel injection cannulae (o.d. = 0.28 mm; i.d. = 0.18 mm) that extended 2 mm beyond the tip of the guide cannulae. Injection cannulae were connected, via polyethylene catheter, to a 1 μl Hamilton microsyringe driven by a programmed Harvard infusion pump. The drug solutions were infused over a 1 min period, the inner cannulae being removed after a further min and replaced by stylets.

The cortical EEG was recorded bilaterally between ipsilateral fronto–parietal electrodes on an electroencephalograph, Alvar, in freely moving animals. After the rat had been habituated to the test cage for 10 min, a 20 min reference EEG was recorded. The drug was then injected and the EEG was recorded continuously for 120 min. During EEG recordings, the animals were observed and kept awake by gentle sensory stimulation if necessary.

The data are expressed as means ± S.E. mean of the cumulated duration of spike-and-wave discharges measured visually per consecutive 20 min periods. A non-parametric statistical analysis for related samples, using the Wilcoxon test, was applied for comparison of responses to each drug regimen versus control condition (solvent injection).

2.5. Convulsive effects

Two groups of 8 GAERS and two groups of 8 NE were used. In each group, all rats were injected i.p. with the different doses of the drug under study (CGP 36742, 800–2400 mg/kg; CGP 56999, 3–6 mg/kg). Procedures for injections and EEG recordings were as above. In each group and for each dose, the main EEG and behavioral features of the convulsive seizures induced by the $GABA_B$ receptor antagonists were characterized by visual analysis. The incidence of convulsions in the two strains were compared using the χ^2 test.

Two groups of 8 NE rats were pretreated with an injection of the $GABA_B$ receptor agonist R-baclofen (4 mg/kg i.p.) 20 min before CGP 36742 (2400 mg/kg i.p.) or CGP 56999 (6 mg/kg i.p.).

2.6. Proconvulsant effects on audiogenic seizures

Each drug was assigned to a group of 10 AS rats. In each group, all rats served as their own controls. The rats were injected i.p. (2 ml/kg) in a random sequence with the different doses of the drug under study (CGP 36742, 100–200 mg/kg; CGP 56999, 0.25–0.75 mg/kg) and with the saline solvent. The successive experiments were separated by one-week intervals. 60 min after the injection, the AS rats were placed in a cylindrical plexiglass chamber (40 cm in diameter × 50 cm in height) and submitted to white noise (10 000–20 000 Hz, 110 dB) produced by a sound generator. The sound stimulation was initiated 30 s after the rat was placed in the chamber and continued until an audiogenic seizure occurred or for a maximum of 60 s. The latencies between initiation of the sound, wild running and tonic convulsion were measured. The results obtained after injections of solvent or drug were compared using Wilcoxon's test for paired samples.

2.7. c-Fos protein expression

Two groups of six NE rats were injected with CGP 36742, 1600 mg/kg, or with CGP 56999, 6 mg/kg. Two control rats were similarly injected with saline. Rats were anaesthetized and decapitated 120 min after the first convulsive seizure, or if no seizure occurred, 150 min after injection. Brains were quickly removed and frozen, and 25 μm thick coronal sections were prepared in a cryostat. These were mounted on chrome–alum gelatin-coated slides, fixed in 4% paraformaldehyde for 5 min and sequentially rinsed in Tris-buffered saline (TBS), TBS containing 0.2% hydrogen peroxide, TBS with 2% bovine serum albumin and finally 0.25% Triton X-100 for 1 h. The immuno-cytochemical procedure consisted of sequential incubation with the rabbit polyclonal antibody against c-Fos (Santa Cruz Biotechnology SC-052, 1:500 dilution) overnight, followed by biotinylated goat anti-rabbit serum (1:400 dilution) for 1 h and by avidin–biotin–peroxidase complex (Vector, 1:25 dilution) for 1 h. After 3 × 10 min washes in TBS, slide-mounted sections were placed for 10 min in diaminobenzidine (0.25 mg/ml), 0.02% hydrogen peroxide and NiCl 0.02%. Sections were finally rinsed and dehydrated. The distribution of positive neurons was determined by light microscopy within anatomically defined

regions of the brain. The density/intensity of labelled cells was subjectively rated on a four-point scale (0 = no labelling; 1 = low; 2 = moderate; 3 = intense labelling) for semi-quantitative evaluation.

2.8. Cortical and hippocampal microinjections

Unilateral cortical or hippocampal infusions were given to freely moving NE rats. The inner cannula, which extended 0.5 mm beyond the guide cannula in the cortex and 1 mm in the hippocampus, was connected via a 50 cm polyethylene catheter to a microsyringe driven by a micropump. The injections were delivered at a rate of 1 μl/30 min until occurrence of a convulsive seizure, or for a maximum of 60 min. At the end of the infusion, the injection cannulae were removed and replaced by stylets.

Six NE rats fitted with bilateral cortical cannulae were unilaterally injected with CGP 36742 (1, 10 and 100 μg/μl per 30 min) or CGP 56999 (1 and 100 μg/μl per 30 min). Two NE rats fitted with bilateral hippocampal cannulae were unilaterally injected with CGP 36742 (1 and 10 μg/μl per 30 min) or CGP 56999 (0.1 and 0.5 μg/μl per 30 min). At least three days separated two infusions in the same rat.

The EEG was continuously recorded from the 4 cortical screw electrodes and from the guide cannulae for 5 h. The animals were constantly observed, and occasionally handled. The number, duration, EEG and behavioral characteristics of convulsive seizures were noted.

2.9. Histological controls

After completion of the microinjections, animals were killed with an overdose of pentobarbitone. The brains were removed, frozen and stored at −70°C. Coronal sections of 20 μm were cut on a cryostat, fixed in paraformaldehyde and stained with cresyl violet. Injection sites were localized with reference to the Atlas of Paxinos and Watson (1986).

3. Results

3.1. Anti-absence action

In GAERS, the spike-and-wave discharges characteristic of absence seizures were dose dependently suppressed following i.p. injection of CGP 36742 (50–400 mg/kg) or CGP 56999 (0.25–0.75 mg/kg). Full suppression was attained with 400 and 0.75 mg/kg respectively within 40 min and lasted several h (Fig. 1). No side-effects were evident at these doses and the background EEG was unaltered.

Bilateral microinjections into the ventrolateral nuclei of the thalamus of CGP 36742 (0.5–1 μg/side) or CGP 56999 (0.1–0.2 μg/side) also dose dependently suppressed spike-and-wave discharges (Fig. 2). No behavioral or EEG changes were noticed after the injections of CGP 36742. In contrast, short-lasting and rare clonic convulsions were induced in 2/8 rats by the highest dose of CGP 56999. Histological examination attested the bilateral location of cannula tracks in the nuclei of the ventrolateral thalamus.

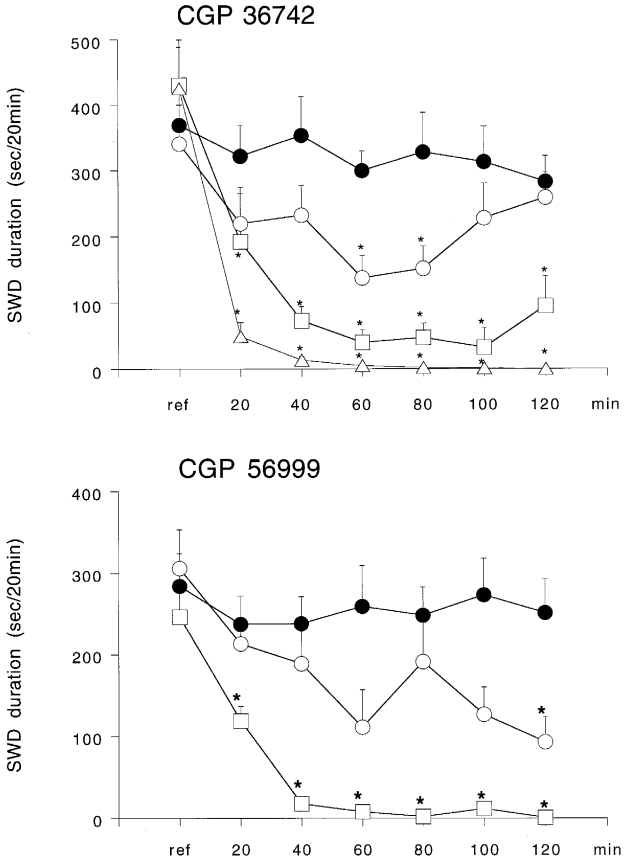

Fig. 1. Dose-dependent suppression of spike-and-wave discharges (SWD) in GAERS, expressed as cumulative duration per sequential 20 min periods, following i.p. injection of CGP 36742 (●, vehicle; ○, 50 mg/kg; □, 200 mg/kg; △, 400 mg/kg) or CGP 56999 (●, vehicle; ○, 0.25 mg/kg; □, 0,75 mg/kg). * $P < 0.01$ versus vehicle.

3.2. Convulsive effects

At high doses, CGP 36742 (≥ 800 mg/kg i.p.) and CGP 56999 (≥ 3 mg/kg i.p.) induced convulsive seizures in GAERS as well as in NE rats; in each group, a similar number of animals had convulsive seizures (Table 1). Convulsive seizures started more than 1 h after the injection and occurred 1–3 times during the 2 h observation period. They were reflected in the EEG by high amplitude polyspike and polyspike-wave discharges lasting 30 to 90 s. Concomitant behavioral manifestations were characterized by clonic contractions of the limbs and of the axial muscles, ranging in intensity from barely perceptible twitches to violent convulsions. Convulsions were often provoked by sensory stimuli (noise, handling of the ani-

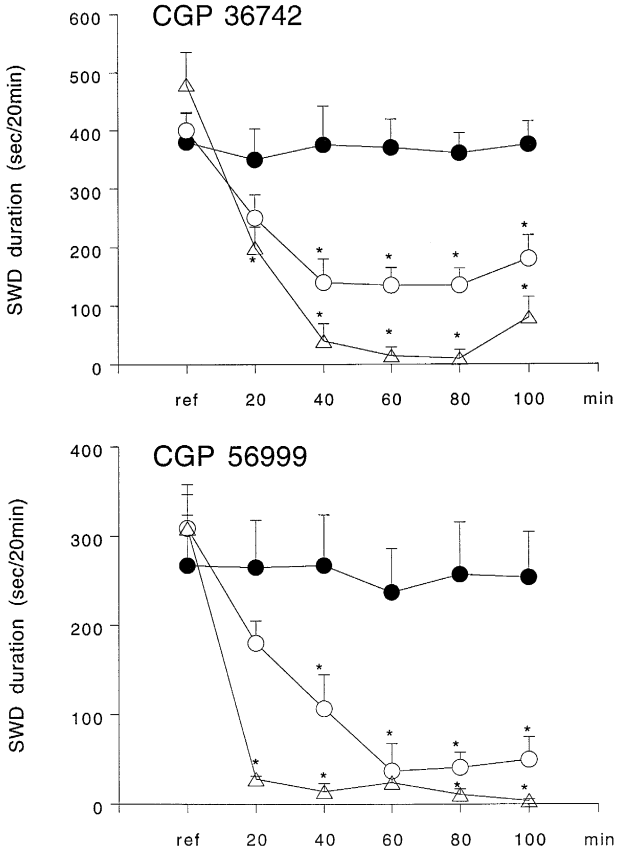

Fig. 2. Dose-dependent suppression of spike-and-wave discharges (SWD) in GAERS following bilateral intrathalamic injection of CGP 36742 (●, vehicle; ○, 0.5 μg/side; △, 1 μg/side) or CGP 56999 (●, vehicle; ○, 0.1 μg/side; △, 0,2 μg/side). * $P < 0.01$ versus vehicle.

mals). In many of the animals, delayed convulsive seizures occurred after the 2 h observation period. They usually appeared shortly after the rats had been removed from the observation cage and returned to their home-cage. In CGP 56999-injected rats a convulsion sometimes occurred as late as 5 h after the injection. Between convulsive seizures, the animals appeared somnolent and remained immobile. The EEG background activity was altered, with numerous bursts of rapid, small amplitude waves.

Administration of the $GABA_B$ receptor agonist baclofen, 4 mg/kg i.p., prevented the convulsant effect of a subsequent 6 mg/kg dose of CGP 56999, or of a 1600 mg/kg dose of CGP 36742, in all of 8 treated NE animals.

The ratio of the dose that completely abolished the spike-and-wave discharges of absence seizures to the dose that provoked convulsions in 50% of the animals varied from 5 to 6 for both compounds.

3.3. Proconvulsant effects on audiogenic seizures

In AS rats, the latencies to elicit the running and the tonic components of audiogenic seizures were dose dependently decreased when the animals were exposed to the inducing sound one hour after injection. The decrease was significant after CGP 36742, 200 mg/kg, and CGP 56999, 0.75 mg/kg (Table 2).

Table 1
Number of GAERS or control NE rats displaying convulsions after i.p. injections of CGP 36742 or CGP 56999

	Drugs (doses mg/kg i.p.)				
	CGP 36742			CGP 56999	
	800	1600	2400	3	6
GAERS	0/8	3/8	4/8	2/8	7/8
NE	1/8	0/8	4/8	3/8	6/8

$n = 8$ in each group.

3.4. C-Fos expression

In this experiment, CGP 36742 (1600 mg/kg i.p.) produced one convulsion in 4/6 NE rats, and CGP 56999 (6 mg/kg i.p.) produced one to five convulsions in 4/6 NE rats. In each group, 2/6 rats did not show any EEG or behavioral convulsive seizures.

Expression of c-Fos in cortical and limbic structures occurred after seizures provoked by i.p. injections of CGP 36742 or CGP 56999. The frontoparietal cortex, piriform and perirhinal cortex, the basolateral and corticomedial nuclei of the amygdala, pyramidal layers of Ammon's horn and granule cells of the dentate gyrus were clearly stained (Table 3, Fig. 3). When no seizures were elicited, both compounds induced only a low expression of c-Fos in the neocortex, the piriform cortex and the amygdala, as well as in the hippocampus after CGP 56999 (Table 3). In all animals, a few stained cells were found in the medial thalamic nuclei and in the central mesencephalic grey. No area was specifically stained in the brain-stem.

In control rats similarly handled and injected with saline, labelled cells were seen in the medial thalamus and mesencephalic central grey. Light labelling occurred in the neocortex, while limbic structures were unstained.

Table 2
Mean latency (s) ± S.E.M. of sound-induced wild running and tonic seizure in audiogenic seizure susceptible rats after i.p. injections of CGP 36742 or CGP 56999

CGP	Dose (mg/kg)	Running	Tonic seizures
36742	0	18 ± 4	27 ± 4
	100	16 ± 5	23 ± 6
	200	8 ± 2 [a]	15 ± 2 [a]
56999	0	20 ± 5	27 ± 4
	0.25	5 ± 3 [a]	15 ± 4
	0.75	4 ± 2 [a]	9 ± 3 [a]

$n = 10$ for each drug.
[a] $P < 0.05$ compared to dose O (Wilcoxon).

Table 3
Local c-Fos protein expression in rats treated with CGP 36742 or CGP 56999

	CGP 36742 (1600 mg/kg i.p.)		CGP 56999 (6 mg/kg i.p.)	
	no seizure ($n = 2$)	seizure ($n = 4$)	no seizure ($n = 2$)	seizure ($n = 4$)
Neocortex	0–1	2 (2–2)	1–1	1.5 (1–2)
Piriform cortex	0–1	2 (2–3)	0–1	2.5 (2–3)
Amygdala	1–1	2 (2–2)	0–1	2 (2–2)
Gyrus dentatus	0–0	3 (3–3)	2–2	3 (3–3)
CA_1	0–0	1 (1–2)	1–1	2 (2–2)
CA_2	0–0	1 (1–2)	0–1	1 (1–1)
CA_3	0–0	1 (1–2)	1–1	2 (2–2)
CA_4	0–0	1 (1–2)	1–1	2 (1–2)

The density of labelled cells is rated from 0 to 3. Results are expressed as medians and ranges.

3.5. Intracortical infusion

Unilateral infusions into the fronto–parietal cortex of CGP 36742, 10 μg/μl per 30 min in 6 animals, first elicited isolated ipsilateral spikes 5 to 16 min after initiation of the infusion. Simultaneously, twitches occurred in the corresponding contralateral innervation area of the body. These spikes progressively increased in amplitude and frequency and became bilateral. After 10 to 30 min, when doses of 3 to 10 μg were injected, a rhythmic spike and spike-and-wave discharge appeared. The discharge, lasting 45 to 100 s, started in the injected area and

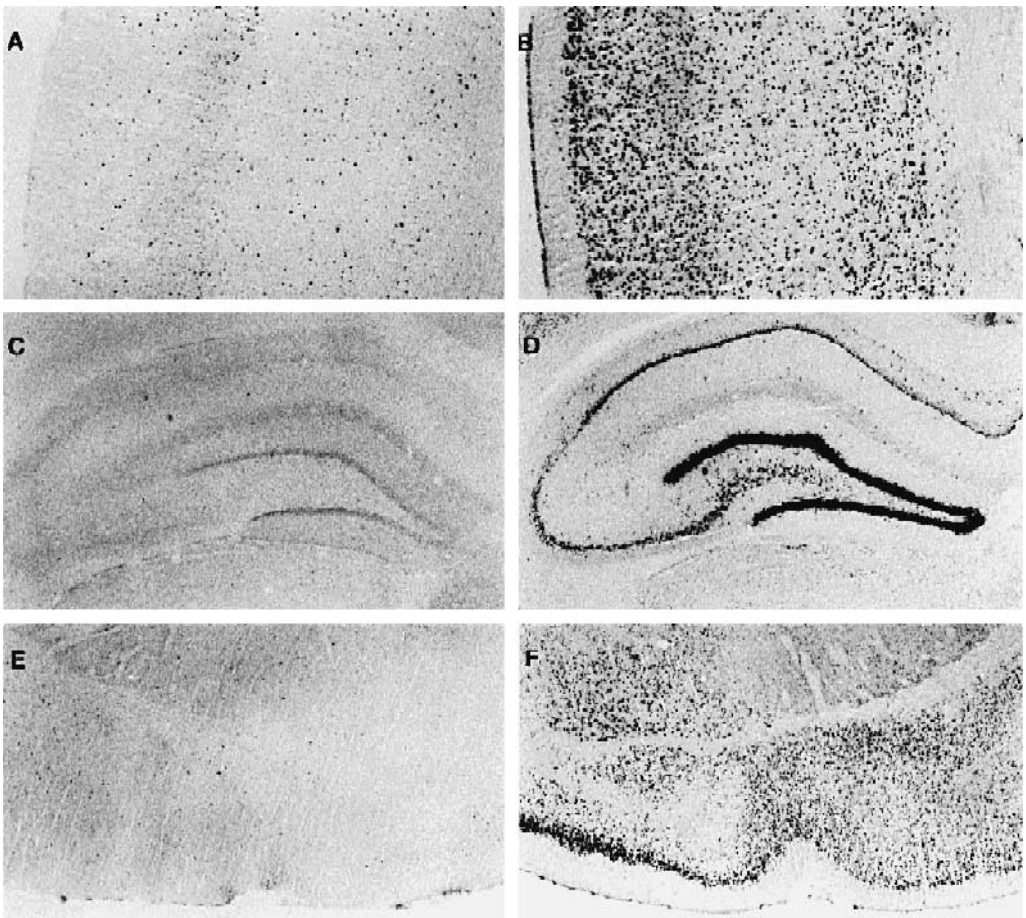

Fig. 3. c-Fos expression in frontoparietal cortex (A,B), hippocampus (C,D), amygdala and perirhinal cortex (E,F) after i.p. injection of CGP 56999, 6 mg/kg, in a rat without seizures (A,C,E) and in a rat with convulsions (B,D,F). c-Fos labeling was low (cortex, hippocampus) or absent (amygdala) in these structures when no convulsions were elicited; it was high after occurrence of seizures.

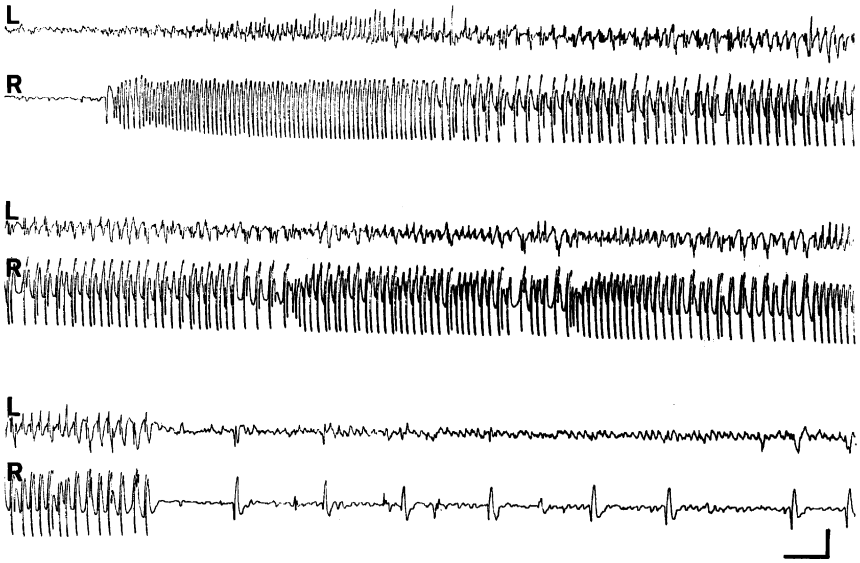

Fig. 4. EEG seizures induced by unilateral infusion of CGP 36742 (10 μg/μl per 30 min) into the right fronto–parietal cortex. L, left cortex; R, right cortex; calibrations, 200 μV, 1 s.

propagated rapidly to the whole ipsilateral and contralateral cortex (Fig. 4). Simultaneously, a clonic seizure developed. After the infusion was stopped, 10–20 seizures per animal occurred for the next 20–40 min. In a further experiment, an infusion rate of 100 μg/μl per30 min was used. After some transient isolated spikes, clonic seizures started between 8 and 12 min after initiation of infusion (25–40 μg). Frequent almost continuous convulsive seizures persisted for 2 to 3 h after the infusion was stopped. Normal EEG and behavior fully recovered after 4 to 5 h. Infusion of 2 μl, at 1 μg/μl per 30 min, failed to induce a convulsion.

Intracortical infusion of CGP 56999 at a rate of 1 μg/μl per 30 min for 60 min in 5 animals induced bilateral clonic seizures starting 20–85 min after the end of the infusion. A total of 4–5 seizures per rat occurred during the 5 h recording period. Seizures were sometimes elicited by handling. A higher concentration (100 μg/μl per 30 min) produced similar results: a few convulsive seizures were observed with a long latency, after the end of the 60 min infusion.

3.6. Hippocampal infusion

Unilateral infusion of the $GABA_B$ receptor antagonists into the hippocampus induced seizures originating in this structure. In two animals, CGP 36742, 10 μg/μl, elicited the first seizure after 20–25 min of infusion when about 7 to 8 μg had been injected. These seizures were characterized by a polyspike-wave discharge lasting 50–90 s, which started in the injected hippocampus and spread rapidly to the contralateral hippocampus and to the cortex. The main discharge was followed, after a 30 s period of apparent EEG normalization, by a recurrent discharge of slow spikes for 15–30 s. During the EEG discharge, the animals were immobile or displayed facial automatisms. Rapid head shakes appeared at the end of the seizure. During the first hour following the interruption of the infusion, two seizures were observed in one rat and nine in the other one. Isolated biphasic spikes were recorded interictally. After 1 h, seizures and interictal spikes disappeared. Infusion of 1 μg/μl per 30 min failed to induce a convulsion.

Similar seizures were elicited by intrahippocampal infusion of CGP 56999, 0.5 μg/μl per 30 min in 2/2 NE rats. After 40 min of infusion (0.7 μg), the first spike and spike-and-wave discharges lasting 25 and 140 s respectively, were recorded. In the two rats, 18 and 23 EEG seizures were observed during the 5 h recording period, sometimes as a consequence of handling. Usually, EEG seizures were not accompanied by any behavioral convulsions, but they frequently ended with rapid head shakes. However, three seizures evolved into tonic–clonic convulsions. No interictal spikes were seen. Infusion of 0.1 μg/μl per 30 min had no effect.

In all cases, histological controls confirmed the correct placement of the injection cannulae in the fronto–parietal cortex or in the hippocampus. Local gliosis was found, around the tip of the cannulae, in the target areas.

4. Discussion

The present results confirm and extend the previously reported suppressant effects of $GABA_B$ antagonists on absence seizures (Liu et al., 1992; Marescaux et al., 1992a,b). As was shown with CGP 35348, systemic injection of CGP 36742 and CGP 56999 suppressed the spontaneous spike-and-wave discharges in rats with genetic absence epilepsy without inducing side-effects. In the present experiments, the efficiency of CGP 36742 and CGP 56999

was grossly related to their respective affinities for the $GABA_B$ receptors: CGP 56999 with an affinity in the nanomolar range, was more potent than CGP 36742, with an affinity in the micromolar range, in all the experiments. However the ratio of the binding to the $GABA_B$ receptors of CGP 36742 and CGP 56999 would predict a higher potency for the latter (see below). The importance of thalamic $GABA_B$ receptors in the generation of absence seizures was confirmed by the dose-dependent suppression of spike-and-wave discharges when $GABA_B$ receptor antagonists were injected into the specific relay nuclei of the thalamus. This effect may be related to the suppression of late inhibitory postsynaptic potentials mediated via activation of post-synaptic $GABA_B$ receptors. These potentials were shown to be involved in the generation of rhythmic activities in the thalamocortical network (Crunelli and Leresche, 1991; Coulter and Zhang, 1994; Crunelli et al., 1994). However, presynaptic $GABA_B$ receptors may also participate in the pathogenesis of absence seizures by selectively modulating the release of GABA and glutamate in the thalamic ventrolateral relay nucleus (Banerjee and Snead, 1995; Hosford et al., 1995; Lin et al., 1995; Crunelli et al., 1996).

These pharmacological data suggest that a dysfunction in $GABA_B$ receptor-mediated transmission may be involved in the generation of spike-and-wave discharges in genetic absence epilepsy. Indeed, in the lethargic mouse model of absence seizures, a selective increase in the number of $GABA_B$ receptors was demonstrated (Hosford et al., 1992, 1995; Lin et al., 1993, 1995). Previous investigations using $GABA_B$ receptor antagonists failed to demonstrate any differences in either affinity or number of $GABA_B$ receptors in the brains of GAERS as compared to NE controls (Knight and Bowery, 1992; Mathivet et al., 1994). However, a more recent study, using a potent $GABA_B$ receptor agonist, indicated that the affinity of agonist binding was greater in a cortical membrane preparation of GAERS (Mathivet et al., 1996). Moreover, recent evidence suggests that there is a raised extracellular level of GABA within the ventrolateral thalamus of GAERS, and this may be sufficient to increase the activation of GABA receptors within the thalamus (Richards et al., 1995).

The present results also demonstrate that $GABA_B$ receptor antagonism provokes convulsive seizures, although only at doses at least 5–6-times higher than the absence-suppressant doses of CGP 36742 and CGP 56999. The two compounds used in the present experiments bind selectively to the $GABA_B$ receptor (Bittiger et al., 1992, 1993) although CGP 36742 has a low affinity for $GABA_A$ receptors ($IC = 500\ \mu M$). Nevertheless, the suppression by baclofen of the convulsant potency of both drugs demonstrates the predominant involvement of $GABA_B$ receptors in this effect.

The fact that a high dose of both $GABA_B$ receptor antagonists had a similar convulsant potency in GAERS and in NE controls shows that this effect is not related to a particular strain-sensitivity to the $GABA_B$ receptor antagonist.

Both $GABA_B$ receptor antagonists were proconvulsive on audiogenic seizures at doses which also suppressed absence seizures. They shortened the latencies for occurrence of wild running and tonic seizures elicited by sound stimulation, suggesting a facilitation of the build up of excitation in brain-stem structures underlying these seizures. Alteration in GABAergic function in the inferior colliculus, which includes $GABA_B$ receptors, has already been shown to modulate susceptibility to audiogenic seizure initiation (Faingold et al., 1994).

The present results are in agreement with previous data obtained with CGP 35348, which shares many pharmacological properties with CGP 36742 (Froestl et al., 1992; Bittiger et al., 1992, 1993; Marescaux et al., 1992b). CGP 35348 is proconvulsant on audiogenic seizures and convulsant at high doses in NE and GAERS (personal observations). It also facilitates convulsions in isoniazid-pretreated mice and elicits epileptic-like discharges in hippocampal slices (Karlsson et al., 1990, 1992; Scanziani et al., 1992, 1994). Microiontophoretic application of CGP 35348 in the cortex of rats led to an increase in the firing rate and in excitatory responses of cortical neurons (Andre et al., 1992).

Given the pharmacological profile of generalized convulsive and non-convulsive seizures, the fact that $GABA_B$ receptor antagonists display simultaneously anti-absence and proconvulsant properties does not seem contradictory. In humans as in animal models, carbamazepine and phenytoin, antiepileptic drugs which are specifically effective against generalized convulsive or focal seizures, potentiate absence seizures; conversely, ethosuximide is a pure anti-absence drug and is ineffective against convulsions (Vergnes and Marescaux, 1994). Similarly, potentiation of $GABA_A$ transmission aggravates clinical and experimental forms of absence seizures and produces absence-like seizures in non-epileptic animals, but decreases the generation of generalized convulsive and partial seizures (Vergnes et al., 1984; Marescaux et al., 1992a,b; Vergnes and Marescaux, 1994; Snead, 1994).

The large increase in c-Fos protein expression in the neocortex, hippocampus, amygdala and surrounding piriform and perirhinal cortex after seizures induced by i.p. injection of high doses of CGP 36742 and CGP 56999 showed that limbic and cortical structures were primarily involved in the induced seizures (Dragunow and Faull, 1989; Kiessling and Gass, 1993). Even when no seizures occurred, some expression of c-Fos was detected in the amygdala after CGP 36742 and in the cortex and hippocampus after CGP 56999, indicating that these structures were already activated by the drugs themselves. In contrast, in saline-injected controls, c-Fos was not expressed in any limbic structure, while clear labelling in the medial nuclei of the thalamus and in the mesencephalic central

grey, as well as weak labelling in the neocortex, expressed the non-specific activation induced by the experimental procedure.

The susceptibility of the cortex and the hippocampus to the epileptogenic effects of the $GABA_B$ receptor antagonists was further confirmed by the effects of local drug application to these structures. Indeed, local microinjections of CGP 36742 and CGP 56999 into the cortex or the hippocampus produced focally initiated seizures which spread rapidly over both hemispheres. Cortically initiated seizures had motor components according to the activated motor areas, whereas hippocampally initiated seizures were similar to limbic motor seizures, unless they generalized into full tonic–clonic fits.

There is a discrepancy between the high ratio (17500) of IC_{50} values of CGP 36742 (35000 nM) and CGP 56999 (2 nM) and the low ratio of their pharmacological potency. For the absence-suppressant effect, the ratio of the suppressant doses of CGP 36742 and CGP 56999 was about 500 with systemic administration and about 10 with intrathalamic injection. For the proconvulsant and convulsant effects the ratio of the effective doses of CGP 36742/CGP 56999 was about 300 after i.p. injections and varied around 10 for intracortical and intrahippocampal injections. The discrepancy between the binding ratio and the pharmacological potency may have several explanations. The $GABA_B$ receptor antagonists have a poor penetration through the blood–brain barrier and a low bioavailability, due to their physico–chemical characteristics (Froestl et al., 1992). The effective amount of the compound binding to the receptors at a given brain site in vivo is thus considerably reduced and delayed over time. Different effects of the two compounds on various types of $GABA_B$ post- and presynaptic receptors (Bonanno and Raiteri, 1993) may also affect their respective potency. However, the ratio of the effective doses in suppressing absences and in producing convulsions was of the same magnitude, in spite of differences in the mechanisms involved, suggesting a reduced access of CGP 56999 to its binding sites.

The convulsions induced by systemic $GABA_B$ receptor antagonists were often characterized by long latencies, commonly exceeding one hour. By contrast, the absence-suppressant effect was obtained within 20–40 min. The differences in sensitivity and in time course of the absence seizure-suppressant and the convulsion-inducing effects of $GABA_B$ receptor antagonists suggest that the two effects may be mediated by different cellular mechanisms. Suppression of spike-and-wave discharges in absence seizures is related mainly to blockade of thalamic pre- and postsynaptic $GABA_B$ receptors (Crunelli and Leresche, 1991; Crunelli et al., 1996). In general, the postsynaptic blockade of the $GABA_B$-mediated late inhibitory potential leads to a shortened period of inhibition (Karlsson et al., 1990, 1992; Andre et al., 1992; Olpe et al., 1992, 1993) which might contribute to increased local excitability. However, the convulsions elicited by $GABA_B$ receptor antagonists may primarily involve presynaptic hetero- and autoreceptors controlling the release of glutamate and GABA from the nerve endings in the cortex and the hippocampus (Bonanno and Raiteri, 1992; Waldmeier et al., 1994).

In conclusion, $GABA_B$ receptors at pre- and postsynaptic sites contribute to the control of neural activity. Blockade of $GABA_B$ receptors within the thalamus should suppress thalamo–cortical oscillations and absence-like spike-and-wave discharges. At higher doses, pre- and post-synaptic mechanisms may combine and the net effect of $GABA_B$ receptor antagonism would be to provoke an imbalance in favor of cortical and limbic excitation, culminating in paroxysmal disorders and convulsive activity.

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